

APPLICATION
FOR
UNITED STATES LETTERS PATENT

TITLE: INHIBITION OF PKC TO TREAT PERMABILITY
FAILURE

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5 This application claims the benefit of a previously filed Provisional Application No. 60/124,043, filed March 12, 1999, the contents of which is incorporated in its entirety.

10 The invention relates to peritoneal dialysis fluids, and methods of making and using them. More particularly, it relates to the methods for preventing and treating complications due to peritoneal dialysis.

Peritoneal dialysis can be used to treat patients having chronic renal failure. In this procedure, peritoneal dialysis fluid is introduced into the peritoneal cavity of a subject. The fluid generally has a high glucose level, often in the 100 mM range. Introduction of this relatively high molarity fluid into the peritoneum causes the osmotic extraction of toxins, for example, urea creatinine, and other substances normally removed by the kidney, from the blood. It can also reduce the level of fluid in the patient.

The invention is based, in part, on the discovery that the use of peritoneal dialysis fluid, especially prolonged use, can result in decreasing permeability between the peritoneal dialysis fluid and the blood compartment, that high levels of glucose are involved in changes in the cells lining the peritoneum, that the high glucose levels
25 activate PKC, and that the inhibition of PKC can prevent this chronic complication. The invention can treat an undesirable decrease in the efficiency of exchange between the peritoneal dialysis fluid and the vascular compartment.

Accordingly, the invention features, a method of treating a subject. The

method includes:

introducing peritoneal dialysis fluid into the peritoneum of the subject; and
inhibiting a PKC in the subject,
thereby treating said subject.

15 In a preferred embodiment, an inhibitor of PKC is included in the peritoneal
dialysis fluid. The inhibitor is preferably a specific inhibitor of PKC. The inhibitor can
be an inhibitor of a PKC β , e.g., $\beta 1$ or $\beta 2$, γ , δ , or other isoform. The inhibitor can be, for
example, a bis (indolyl) maleimide, for example, the PKC β inhibitor LY333531, which
is described in Ishi et al. (1996) *Science* 272:728-731, hereby incorporated by reference.
10 LY333531 can be present in the dialysis fluid at about 1-1,000, 5-750, 20-500, but more
preferably 50-500 nanometers.

In a preferred embodiment, the concentration of glucose in the peritoneal
dialysis fluid is 200nM.

Peritoneal dialysis fluid of the invention is particularly useful for subjects who
15 are already peritoneal dialysis patients. In preferred embodiments, the subject has been a
peritoneal dialysis patient for at least 2, 4, 6, 12, or 24 months, e.g., the subject has been
administered peritoneal dialysis fluid, either one with or without a PKC inhibitor,
periodically for at least 2, 4, 6, 12, or 24 months, or it has been at least 2, 4, 6, 12, or 24
months since the subjects first peritoneal dialysis administration. Peritoneal dialysis fluid
20 of the invention can be administered to patients who have already developed permeability
disjunction.

In other embodiments peritoneal dialysis fluid of the invention is useful for
subjects who have not yet had peritoneal dialysis. Peritoneal dialysis fluid of the
invention can be administered to patients who have not yet developed permeability
25 disjunction.

In another aspect, the invention features, a method of treating a subject. The
method includes:

introducing peritoneal dialysis fluid and an inhibitor of a PKC into the subject,

thereby treating the subject.

In a preferred embodiment, the peritoneal dialysis fluid and PKC inhibitor are co-administered to the subject; the peritoneal dialysis fluid and PKC inhibitor are introduced separately into the subject; the peritoneal dialysis fluid and PKC inhibitor are
5 combined prior to introduction into the subject and administered together.

~~In a preferred embodiment, an inhibitor of PKC is included in the peritoneal dialysis fluid. The inhibitor is preferably a specific inhibitor of PKC. The inhibitor can be an inhibitor of a PKC β , e.g., $\beta 1$ or $\beta 2$, γ , δ , or other isoform, or combinations thereof. The inhibitor can be, for example, a bis (indolyl) maleimide, for example, the PKC β inhibitor LY333531. LY333531 can be present in the dialysis fluid at about 1-1,000, 5-750, 20-500, but more preferably 50-500 nanometers.~~

In a preferred embodiment, the concentration of glucose in the peritoneal dialysis fluid is 200nM.

In a preferred embodiment, the subject is administered a second, third, fourth,
15 or fifth, infusion of peritoneal dialysis fluid.

Peritoneal dialysis fluid of the invention is particularly useful for subjects who are already peritoneal dialysis patients. In preferred embodiments the subject has been a peritoneal dialysis patient for at least 2, 4, 6, 12, or 24 months, e.g., the subject has been administered peritoneal dialysis fluid, either one with or without a PKC inhibitor,
20 periodically for at least 2, 4, 6, 12, or 24 months, or it has been at least 2, 4, 6, 12, or 24 months since the subjects first peritoneal dialysis administration. Peritoneal dialysis fluid of the invention can be administered to patients which have already developed permeability disjunction.

In other embodiments, peritoneal dialysis fluid of the invention is useful for
25 subjects who have not yet had peritoneal dialysis. Peritoneal dialysis fluid of the invention can be administered to patients which have not yet developed permeability disjunction.

In a preferred embodiment, the subject is at risk for renal failure, for example, the subject is a patient in end-stage renal failure.

In another aspect, the invention features, a peritoneal dialysis fluid which includes an inhibitor of a PKC.

In a preferred embodiment, the peritoneal dialysis fluid is one described
5 herein.

In a preferred embodiment, an inhibitor of PKC is included in the peritoneal dialysis fluid. The inhibitor is preferably a specific inhibitor of PKC. The inhibitor can be an inhibitor of a PKC β , e.g., $\beta 1$ or $\beta 2$, γ , δ , or other isoform, or combinations thereof. The inhibitor can be, for example, a bis (indolyl) maleimide, for example, the PKC β
10 inhibitor LY333531. LY333531 can be present in the dialysis fluid at about 1-1,000, 5-750, 20-500, but more preferably 50-500 nM.

In a preferred embodiment, the concentration of glucose in the peritoneal dialysis fluid is 200nM.

15 In yet another aspect, the invention features, a method of making an improved peritoneal dialysis fluid. The method includes, providing a peritoneal dialysis fluid and adding to that fluid an inhibitor of a PKC, for example an inhibitor described herein, for example LY333531.

In a preferred embodiment, an inhibitor of PKC is included in the peritoneal
20 dialysis fluid. The inhibitor is preferably a specific inhibitor of PKC. The inhibitor can be an inhibitor of a PKC β , e.g., $\beta 1$ or $\beta 2$, γ , δ , or other isoform, or combinations thereof. The inhibitor can be, for example, a bis (indolyl) maleimide, for example, the PKC β inhibitor LY333531. LY333531 can be present in the dialysis fluid at about 1-1,000, 5-750, 20-500, but more preferably 50-500 nM.

25 In a preferred embodiment, the concentration of glucose in the peritoneal dialysis fluid is 200nM.

Subject, as used herein, can refer to a human subject, or a non-human animal, for example, a horse, cow, goat, pig, sheep or other veterinary, food or fiber producing

animal, in need of dialysis. The subject can be an individual at risk for (e.g., the individual can have or be predisposed to have) end-stage renal disease, from any cause.

Other features and advantages of the invention will be apparent from the description herein and from the claims.

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Detailed Description of the Drawings

Figure 1 shows PKC activity of primary human mesothelial cells at various glucose levels (i.e., low glucose, 60 mM or 150 mM glucose). The PKC activity was determined
10 in the presence or absence of PMA, an activator of PKC.

Figure 2 shows the effect of treatment with vitamin E on PKC activity of human primary mesothelial cells. The mesothelial cells were exposed to low or high (150 mM) levels of glucose in the presence or absence of PMA, an activator of PKC, and the presence or
15 absence of vitamin E, a PKC inhibitor.

Figure 3 shows PKC activity of primary human mesothelial cells cultured in low glucose concentrations, high glucose concentrations (i.e., 60 mM or 150 mM), or mannitol. The PKC activity was determined in the presence or absence of PMA, an activator of PKC.
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Figure 4 shows DAG activity of transformed mesothelial cells at low glucose levels (5.5 mM) and high glucose levels (60 mM).

Detailed Description

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Peritoneal dialysis for chronic renal failure is used by some patients with renal failure. The fluid for peritoneal dialysis uses glucose at 400-mM range. While not wishing to be bound by theory, it appears that at this concentration level, glucose causes complications which can lead to decreasing permeability between the peritoneal dialysis
30 fluid and the blood compartment. The elevation of high glucose causes changes in the

cells lining the peritoneum. The invention is based, in part on, PKC activation, which is believed to cause this chronic decrease in the exchange between the peritoneal fluid and a vascular compartment.

PKC activation induced by hyperglycemia either in the peritoneal fluid or in
5 the blood causes cells to behave abnormally. The inhibition of PKC β isoform by the inhibitor LY333531 can prevent many of the vascular changes induced by glucose levels up to 20-30mM. The higher concentration of glucose as used in peritoneal dialysis will also increase PCK activation. Inhibition of PKC activation using a PKC β , e.g., $\beta 1$ or $\beta 2$, γ , δ , or other isoform, or combinations thereof, inhibitor will prevent the thickening and
10 the decrease in exchange between peritoneal and vascular compartments, which will improve the efficiency of peritoneal dialysis for chronic renal failure.

As shown in Figures 1-3, exposure of human mesothelial cells, which are the cells that line the peritoneum, to glucose levels of 60 mM or 150 mM results in an
15 increase of PKC activity. These concentrations of glucose are standard for dialysis. In addition, as shown in Figure 2, exposure of these cells to vitamin E, which is an inhibitor of PKC, resulted in a decrease of PKC activity. It was also found that high glucose levels of 60 mM or 150 mM increased DAG activity. These results demonstrate that glucose concentrations used for peritoneal dialysis activate the DAG-PKC pathways. It has been
20 reported that activation of these pathways increases fibrosis. These results also show that inhibitors of PKC such as vitamin E decrease PKC activity. Therefore, inhibition of PKC can decrease fibrosis and reduce or prevent permeability failure of the peritoneal membrane.

Other embodiments are within the following claim.

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